

NTP Research Concept: β -*N*-Methylamino-L-alanine

Project Leader

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Background and Rationale

The nonprotein amino acid, β -*N*-methylamino-L-alanine (L-BMAA) was nominated for toxicological characterization by the National Institute for Environmental Health Sciences due to its potential widespread distribution in the environment, possible presence in certain dietary supplements, and evidence that the chemical is neurotoxic (<http://ntp.niehs.nih.gov/go/33220>). L-BMAA is produced by members of all known groups of cyanobacteria (blue-green algae), including symbiotic and free-living cyanobacteria present in terrestrial, freshwater, brackish, and marine environments. Blue-green algae is used as a dietary supplement and has been previously nominated for toxicity testing by the NTP due to concerns that the commercially available products may contain microcystins and other cyanotoxins (<http://ntp.niehs.nih.gov/go/TS-M010004>). It is uncertain if these products contain L-BMAA. Humans may be exposed to high concentrations of L-BMAA in plants, fish, or animals used as food and/or in water as the result of formation from harmful algae blooms (HABs). Freshwater HABs, mostly attributable to cyanobacteria, have dramatically increased throughout the world, including the U.S., due in large part to perturbations of ecosystems by human actions. Exposure to L-BMAA is of specific concern because the chemical causes degenerative changes to neuronal cells in culture and *in vivo*.

L-BMAA is similar in structure to β -*N*-oxalylamino-L-alanine (L-BOAA), a neurotoxic amino acid found in chickling peas and linked to lathyrism, a paralytic disease associated with degenerative changes in the spinal cord in humans and domesticated animals. In a comparative study, L-BOAA was shown to be more toxic than L-BMAA to cultured mouse cortical neurons, with an ED₅₀ of approximately 20 μ M versus 1 mM for L-BMAA. However, L-BMAA may be more potent than previously reported; cell death of cultured mouse cortical neurons was potentiated by concentrations as low as 10 μ M and concentrations as low as 30 μ M caused death of motor neurons in cultured mouse spinal cord. L-BMAA injected intraperitoneally caused acute neurotoxicity in young chicks (inability to stand) and in rats (weakness and convulsions). Subcutaneous injections of L-BMAA to neonatal rats caused changes in motor function and spinal cord neurochemistry. Intracranial injections of L-BMAA caused death of hippocampal neurons in mice and damage to the substantia nigra in rats. Male cynomolgus monkeys receiving daily oral doses of up to 315 mg/kg/day for 12 weeks, exhibited neurotoxicity characterized by limb tremor and weakness, behavioral changes, parkinsonian features, and degeneration of neurons in the motor cortex and spinal cord. L-BMAA has been detected in brain tissue of humans exhibiting neurological disease and has been suggested to be the causal factor of high incidences of neurotoxicity in specific human populations. The mechanism of action for L-BMAA involves excitotoxic activation of glutamate receptors following formation of a β -*N*-carboxy adduct. Evidence indicates

that L-BMAA is an agonist for both ionotropic (NMDA/AMPA/kainate subtypes) and metabotropic (mGluR5 subtype) glutamate receptors. A study conducted in rats demonstrated facilitated transport of low concentrations of L-BMAA into brain following either iv or oral administration of high doses, with subsequent rapid elimination from the tissue. However, it is likely that only free L-BMAA was detected in the study and potentially bound L-BMAA was not accounted for. Recent work has detected high concentrations of L-BMAA incorporated into the proteins of tissues of plants, animals, and some humans following putative environmental exposure to the chemical.

Key Issues

Humans may be at risk for neurotoxicity due to exposure to L-BMAA in the environment or consumer products. Determining the extent of exposure, potential for bioaccumulation, and the toxic potential of L-BMAA are key issues for assessing potential health risks. A proposed mechanism of neurotoxicity in humans is based on the hypothesis that following exposure, L-BMAA accumulates in proteins of the nervous system, is persistent, is slowly released following protein catabolism over time, and results in long-term exposure and recurrent neurological damage by continuous activation of glutamate receptors. A key issue identified for study by the NTP is the need to assess the proposed mechanism of neurotoxicity in an animal model in order to determine its relevance to humans. Key to this is better understanding of the biological fate of L-BMAA, with emphasis on quantitating bioaccumulation, protein binding, and persistence of L-BMAA in tissues over time. L-BMAA may be a more potent toxin than previously reported; therefore, an additional evaluation of the biological activity of L-BMAA is warranted.

Proposed Research Program

The main goal of this research program is to provide data to further the toxicological evaluation of L-BMAA. The specific aims of the research program are to:

1. Provide data describing internal dose and elimination kinetics for use in characterizing the mechanism(s) of L-BMAA-induced toxicity. These studies will quantitate absorption of ^{14}C L-BMAA following oral administration of single and multiple doses, identify reactive metabolite(s), assess the distribution and accumulation of bound and free L-BMAA-derived material, and follow the elimination of ^{14}C from brain and other tissues over time.
2. Assess the biological activity of L-BMAA using *in vitro* assays, with emphasis on categorizing effects with known neurotoxicants, including, but not limited to, other glutamate receptor agonists.
3. Analyze for the presence of L-BMAA in samples of blue-green algae dietary supplements.

Significance and Expected Outcome

Conducting the proposed studies will provide metabolism and disposition data needed for assessing the validity of the proposed mechanism of neurotoxicity and its relevance

to humans, further characterize the biological activity of L-BMAA, and determine whether dietary supplement products are a significant source of exposure to L-BMAA. Results of these studies will be used for assessing risk associated with exposure to L-BMAA, for public health guidance, and for determining safe exposure limits to humans.

Selected References

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